COMBINATION OF NO SYNTHASE INHIBITOR(S) AND METABOLIC ANTIOXIDANT(S)

The invention relates to a pharmaceutical composition containing, as active ingredient, one or many substance(s) which interfere(s) with the synthesis of nitrogen monoxide by inhibition of NO synthase and one or many metabolic antioxidant(s) which intervene(s) in the redox status of thiol groups, and optionally a pharmaceutically acceptable support. The invention also relates to a product containing one or many NO synthase inhibitory substance(s) and one or many metabolic antioxidant(s) which intervene(s) in the redox status of thiol groups, as a combination product, in separated form, of these active ingredients.

A pharmaceutical composition and a product according to the invention are useful in the treatment of pathologies where nitrogen monoxide and the metabolism of antioxidants (such as vitamin E or glutathione) as well as the redox status of the thiol groups are involved, and in particular :

. cardiovascular and cerebrovascular disorders comprising, for example, migraine, arterial hypertension, cardiac or cerebral infarctions of ischemic or haemorragic origin, ischemias and thromboses ;

. septic shock, radioactive irradiation, solar radiation, organ transplants ;

. disorders of the central or peripheral nervous system such as, for example, neurodegenerative diseases where cerebral infarctions, senile dementia, including Alzheimer's disease, Huntington's chorea, Parkinson's disease, Creutzfeld-Jacob's disease, prion diseases, amyotrophic lateral sclerosis, but also pain, cerebral or bone marrow traumas, addiction to opiates, alcohol and addictive substances, erective and reproductive disorders, cognitive disorders, encephalopathies, depression, anxiety, schizophrenia, epilepsy, sleeping disorders, eating disorders (anorexia, bulimia, etc.) can be mentioned in particular ;

. proliferative and inflammatory diseases such as, for example, cancer, atherosclerosis, pulmonary hypertension, glomerulonephritis, portal hypertension, cataracts, psoriasis, arthrosis and rhumatoid arthritis, fibroses, amyloidoses, inflammations of the gastrointestinal system (colitis, Crohn's disease) or of the pulmonary system and airways (asthma, sinusitis) as well as contact or delayed hypersensitivities ;

. auto-immune and viral diseases such as lupus, AIDS, parasitic and viral infections, diabetes and its complications including retinopathies, nephropathies and polyneuropathies, multiple sclerosis, myopathies ;

. autosomal genetic diseases such as Unverricht-Lundborg disease ;

. pathologies characterized by a production or a dysfunction of nitrogen monoxide and/or the metabolism of glutathione and of the redox status of thiol groups.

In all these pathologies, there is experimental evidence demonstrating the involvement of nitrogen monoxide or of a dysfunction of the metabolism of glutathione (Kerwin et al., Nitric oxide : a new paradigm for second messengers, J. Med. Chem. 38, 4343-4362, 1995 ; Packer et al., Alpha-lipoic acid as biological antioxidant, Free Radical Biology & Medicine 19, 227-250, 1995). This is the case in particular in Parkinson's disease which illustrates the invention (Beal MF, Excitotoxicity and nitric oxide in Parkinson's disease pathogenesis. Ann. Neurol. 44[Suppl 1], S110-S114, 1998 ; Donato et al., Gluthathione in Parkinson's disease : a link between oxidative stress and mitochondrial damage. Ann. Neurol. 32, S111-S115, 1992). In this context, medicaments which can inhibit the formation of nitrogen monoxide and/or re-establish the biological functionality of the thiol groups or glutathione can have beneficial effects. As is shown in the experimental part, combining an NO synthase inhibitor and a metabolic antioxidant, active ingredients acting with different mechanisms, increases the therapeutic effect of these active ingredients in unexpected fashion. This invention is particularly well illustrated in an experimental pathological model of Parkinson's disease : intoxication with MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydro-pyridine).

A subject of the invention is therefore a pharmaceutical composition containing, as active ingredient, one or many substance(s) which interfere(s) with the synthesis of nitrogen monoxide by inhibition of NO synthase and one or many metabolic antioxidant(s) possessing at least two thiol groups and which intervene(s) in the redox status of thiol groups, and optionally a pharmaceutically acceptable support.

A more particular subject of the invention is a pharmaceutical composition containing, as active ingredient, a substance which interferes with the synthesis of nitrogen monoxide by inhibition of NO synthase and a metabolic antioxidant which intervenes in the redox status of thiol groups.

The term NO synthase inhibitor should be understood to mean any specific or non-specific inhibitor of one of its isoforms, either constitutive (neuronal or

endothelial) or inductible (Kerwin et al., Nitric oxide: a new paradigm for second messengers, J. Med. Chem. 38, 4343-4362, 1995). NO synthase inhibitors according to the invention can be chosen, for example, from certain amino acid derivatives such as L-arginine derivatives, guanidines, isothioureas, nitro- or cyano-aryls, amino-pyridines or amino-pyrimidines, amidines, indazoles or imidazoles as defined hereafter.

The term metabolic antioxidant substance which intervenes in the redox status of thiol groups should be understood to mean any chemical substance possessing at least two thiol groups capable of forming an intra or intermolecular disulphide bridge by oxidation, this substance being able to be found in reduced or oxidized form. Such compounds allow the chelation of divalent cations, the regeneration of antioxidants such as vitamin E or glutathione, and intervene in the redox status of thiol groups. The metabolic antioxidants according to the invention can be chosen, for example, from dithiothreitol, pyritinol, lipoic acid (Packer et al., Alpha-lipoic acid as biological antioxidant, Free Radical Biology & Medicine 19, 227-250, 1995) or its derivatives as defined hereafter, the dimeric disulphide derivatives of penicillamine or N-acetylcysteine, or also the peptides containing at least two cysteine residues. These substances can be natural or synthetic.

In a pharmaceutical composition according to the invention, the NO synthase inhibitor and metabolic antioxidant can be present in separated form or in combined form forming a salt. Of course, the formation of a salt is only envisaged if one of the active ingredients has an acid group and the other active ingredient a basic group. Preferably, the salt is formed from a derivative of the NO synthase inhibitory substance containing at least one basic group and a derivative of the metabolic antioxidant containing an acid group. Thus, the NO synthase inhibitor can be chosen, for example, from the compounds as defined hereafter. The metabolic antioxidant can be chosen, for example, from lipoic acid or its derivatives as defined hereafter, the dimeric disulphide derivatives of penicillamine or N-acetylcysteine.

A subject of the invention is also a product containing one or many NO synthase inhibitory substance(s) and one or many metabolic antioxidant substance(s) possessing at least two thiol groups, which intervene(s) in the redox status of thiol groups, as combination product, in separated form, for simultaneous or sequential use in the treatment of pathologies in which nitrogen monoxide and/or the redox status of thiol groups are involved, such as cardiovascular and cerebrovascular disorders, septic shock, radioactive irradiation, solar radiation, organ transplants, disorders of the central or peripheral nervous system and more particularly

Parkinson's disease, proliferative and inflammatory diseases, autoimmune and viral diseases, diabetes and its complications, autosomal genetic diseases and all the pathologies characterized by a production or a dysfunction of nitrogen monoxide and/or involving the redox status of thiol groups.

In a pharmaceutical composition or product according to the invention, the NO synthase inhibitor and the metabolic antioxidant can be present in doses which can be identical or different. The doses are chosen according to the compounds combined with appropriate diluents or excipients.

The NO synthase inhibitor and metabolic antioxidant can be administered in simultaneous or sequential manner, by the same administration route or by different routes, according to whether they are present in separated or combined form. Preferably, the administration routes are oral, parenteral or topical.

Among NO synthase inhibitors, compounds of amino-acid type, non amino-acid type and aromatic amine type can be defined. NO synthase inhibitors of amino-acid type can be compounds as described in the Applications WO 95/00505, WO 94/12163, WO 96/06076, WO 98/28257, or L-arginine, ornithine, or lysine derivatives as described in the Applications WO 93/24126, WO 95/01972, WO 95/24382, WO 95/09619 and WO 95/22968 (the amino acids are excluded from this class as they have no activity in the NO system ; L-arginine alone has an activity : this is the natural substrat of NO synthase).

NO synthase inhibitors of non amino-acid type can be compounds of the guanidine, isothiourea, nitro- or cyano-aryl, amino-pyridine or amino-pyrimidine, amidine, indazole or imidazole families as well as substituted heterocycles or condensed piperidines.

NO synthase guanidine inhibitors can be compounds as defined in the Applications WO 95/28377, WO 91/04023, WO 94/21621, WO 96/18607 and WO 96/18608.

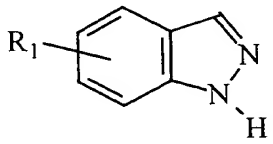
NO synthase isothiourea inhibitors can be compounds as defined in the Applications WO 95/09619, WO 96/09286, WO 94/12165, WO 96/14842, WO 96/18607, WO 96/18608, WO 96/09286, EP 717040 and EP 718294.

NO synthase nitro- or cyano-aryl inhibitors can be compounds as defined in the Application WO 94/12163.

NO synthase amino-pyridine or amino-pyrimidine inhibitors can be compounds as defined in the Applications WO 94/14780, WO 96/18616, WO 96/18617, WO 98/45294, WO 98/24766, WO 00/02860, JP 98/001470, JP 98/120654 and JP 98/036351.

NO synthase amidine inhibitors can be compounds as defined in the Applications WO 95/11014, WO 96/01817, WO 95/05363, WO 95/11231, WO 96/14844, WO 96/19440, WO 98/42696, WO 98/58934, WO 98/50380, WO 98/50382, JP 98/265450, or compounds such as N-phenyl-2-thiophenecarboximidamide.

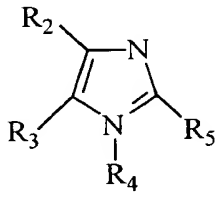
NO synthase indazole inhibitors can be compounds as defined in the Application WO 98/02442 or compounds of general formula I_A



I_A

in which R_1 represents one or more substituents chosen from a hydrogen atom, the nitro, halo, lower alkyl or lower alkoxy radical.

NO synthase imidazole inhibitors can be compounds of the general formula II_A



II_A

in which R_2 and R_3 represent, independently, a hydrogen atom, halo, hydroxy, amino, alkyl or alkoxy radical, or R_2 and R_3 are linked together and form the phenyl radical condensed with the imidazole ring, the phenyl radical being optionally substituted by one or more substituents chosen from hydroxy, trifluoromethyl, halo, carboxy, lower alkyl, lower alkoxy or lower alkenyl radicals ; R_4 represents a hydrogen atom, a lower alkyl, amino, lower alkyl amino or phenyl radical, the phenyl radical being optionally substituted by one or more substituents chosen from hydroxy, trifluoromethyl, halo, carboxy, lower alkyl, lower alkoxy or lower alkenyl radicals ; R_5 represents the hydrogen atom, a lower alkyl, amino, lower alkyl amino radical.

As it is used here, the term lower with reference to the alkyl and alkoxy groups designates saturated aliphatic hydrocarbon groups, linear or branched, containing 1 to 6 carbons such as, for example, methyl, ethyl, propyl, isopropyl, butyl, t-butyl, methoxy and ethoxy. With reference to the alkenyl groups, the term lower designates groups containing 2 to 6 carbon atoms and one or more double or triple bonds such as, for example, vinyl, allyl, propenyl, isopropenyl, pentenyl, butenyl, hexanyl, propenyl and butadienyl groups. The term halo designates chloro, bromo, iodo or fluoro.

The condensed piperidines can be compounds as defined in the Application EP 870763.

The substituted heterocycles can be compounds as defined in the Applications WO 98/50372, WO 98/42667, WO 98/46611, WO 99/05131, WO 99/01455, JP 98/182618.

Preferably, the NO synthase inhibitor is a compound of amino-acid type and more particularly L-arginine, ornithine or lysine derivatives, or a compound of the guanidine, isothiourea, nitro- or cyano-aryl, amino-pyridine or amino-pyridine, amidine, indazole or imidazole families.

The metabolic antioxidant can be chosen from dithiothreitol, pyritinol, the compounds as defined in the Application EP 381439, lipoic acid (in racemic or enantiomeric form) and its derivatives, the dimeric disulphide compounds of penicillamine or N-acetylcysteine, and the peptides comprising at least two cysteine residues. Preferably, the derivatives of lipoic acid are the compounds as defined in the Applications EP 855396, EP 236929, EP 869126, FR 2707983, WO 99/45922 and JP 94227979.

A more particular subject of the invention is a composition or a product as defined above, characterized in that the NO synthase inhibitor is chosen from L-nitro-arginine (LNA), L-nitro-arginine methyl ester (LNAME), L-N-monomethylarginine (LNMMA), aminoguanidine, agmatine, 2-amino-1-(methylamino)benzimidazole, 5-nitro-indazole, 6-nitro-indazole, 7-nitro-indazole, 1,2-(trifluoromethylphenyl) imidazole (TRIM), 2-amino-4-methyl-6-(2-aminoethyl)pyridine, 2-iminopiperidine, 2-iminohomopiperidine, 2-imino-5,6-dihydro-1,3-thiazine, 2-imino-5,6-dihydro-1,3-oxazine, N-phenyl-2-thiophenecarboximidamide, 2-iminotetrahydropyrimidine, S-ethylisothiourea, S-methyl-L-thiocitrulline or S-ethyl-L-thiocitrulline.

A more particular subject of the invention is a composition or a product as defined above, characterized in that the metabolic antioxidant is lipoic acid, in racemic or enantiomeric form.

More preferably, a subject of the invention is also a composition or a product as defined above, characterised in that the NO synthase inhibitor is an inhibitor of the neuronal and/or inductible NO synthase.

NO synthase inhibitor compounds and metabolic antioxidants are commercially available or can be prepared by methods known to the person skilled in the art (or by analogy to the latter) (P. Hamley et al, Bioorganic and medicinal chemistry letters, Vol. 5 (15), 1573-1576 (1995) ; W. M. Moore et al, J. Med. Chem., 39, 669-672 (1996) ; E. P. Garvey et al., The Journal of Biological Chemistry, Vol.269 (43), 26669-26676 (1994)).

All the technical and scientific terms used in the present text have the meanings known to a person skilled in the art. Moreover, all patents (or patent applications) as well as other bibliographical references are incorporated by way of reference.

The following examples are presented to illustrate the above procedures and must in no case be considered as a limit to the scope of the invention.

EXPERIMENTAL PART :

Pharmacological study of the products of the invention

The activity of the compounds of the invention was evaluated *in vivo* on a model of neurotoxicity with MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine). The administration of MPTP produces a syndrome similar to Parkinson's disease resulting in a degeneration of the dopaminergic nigrostriatal neurons. This was observed in man, primates and mice [Langston JW and Ballard PA, Parkinson's disease in a chemist working with 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine, N.Engl.J.Med. 309, 310 (1983) ; Burns RS et al., A primate model of parkinsonism : selective destruction of dopaminergic neurons in the pars compacta of the substantia nigra by N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, Proc. Natl. Acad. Sci. U.S.A. 80, 4546-4550 (1983), Heikkila, RE. et al., Dopaminergic neurotoxicity of 1-methyl-4-phenyl-1,2,5,6- tetrahydropyridine in mice, Science, 224, 1451-1453 (1984)].

Mice (C57BL6) weighing 15-25 g are injected three times with 15-20 mg/kg of MPTP by intraperitoneal route at 2-hour intervals. The products are injected by oral

route 90 minutes before each injection of MPTP and 90 min after the last and 24 hours after the first injection of MPTP. The mice are sacrificed 24 hours after the last injection of MPTP. The striatum is removed and its dopamine level is measured by high-performance liquid chromatography coupled with electrochemical detection. The effectiveness coefficient of the compounds is calculated according to the ratio : dopamine level of the product group + MPTP / dopamine level of the MPTP group only. A product for which the effectiveness coefficient is ≥ to 1.5 is considered beneficial.

Let A be the NO synthase inhibitor and B the metabolic antioxidant.

Example 1

Compound AB, combination of the active ingredients A and B. Compound A : N-phenyl-2-thiophenecarboximidamine, powerful NO synthase inhibitor. Compound B : reduced form of lipoic acid, metabolic antioxidant.

Compound of Example 1 : 4 groups of animals are constituted as follows :

Group 1 : treated with MPTP.
Group 2 : treated with A (3 mg/kg) + MPTP.
Group 3 : treated with B (10 mg/kg) + MPTP.
Group 4 : treated with AB + MPTP.

Group No.	Dopamine level ng/mg of tissue	Effectiveness coefficient
1	3.24	-
2	3.77	1.16
3	3.81	1.17
4	5.21	1.60

The results show that the lipoic acid, in reduced form, used as metabolic antioxidant at the dose of 10 mg/kg is ineffective for protecting the animal against the fall in dopamine which occurs after injection of MPTP. The N-phenyl-2-thiophenecarboximidamine used as NO synthase inhibitor at the dose of 3 mg/kg is

also ineffective. In contrast, the combination of the two compounds proves effective in restoring the dopamine level of the animals subjected to the MPTP neurotoxicity.

Example 2

Compound AB, combination of the active ingredients A and B. Compound A : N^Gnitro-arginine, powerful inhibitor of constitutive and inductible NO synthases. Compound B : reduced form of lipoic acid, metabolic antioxidant.

Compound of Example 2 : 4 groups of animals are constituted as follows :

Group 1 : treated with MPTP.
Group 2 : treated with A (3 mg/kg) + MPTP.
Group 3 : treated with B (10 mg/kg) + MPTP.
Group 4 : treated with AB + MPTP.

Group No.	Dopamine level ng/mg of tissue	Effectiveness coefficient
1	4.11	-
2	6.98	1.69
3	4.48	1.09
4	8.65	2.1

The N^Gnitro-arginine used as an inhibitor of NO synthases, effective at the dose of 3 mg/kg, has an increased effectiveness when it is combined with lipoic acid.

The experimental results of Examples 1 and 2 therefore show a potentializing effect, even a synergy between the two types of compounds.